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CASE REPORT

X-linked Liver Glycogenosis in a Taiwanese Family: Transmission From Undiagnosed Males

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X-linked liver glycogenosis (XLG), also known as glycogen storage disease type-IXa, is characterized by hepatomegaly, abnormal liver functions and growth retardation. It is caused by mutations in the *PHKA2* gene that encodes the α-subunit of phosphorylase kinase (PHK). XLG can be divided into two subtypes: XLG-I, with a deficiency in PHK activity in peripheral blood cells and the liver; and XLG-II, with normal PHK activity *in vitro*. This report describes two boys who presented with hepatomegaly and abnormal liver function. Pedigree analysis revealed them to be fifth-degree relatives, with the disease transmitted through undiagnosed grandfathers. Liver histology confirmed GSD diagnosis, and both cases had a deficiency in PHK activity in red blood cells and liver tissues. This is the first report of XLG-I in the ethnic-Chinese population in Taiwan. This report indicates that XLG may be undiagnosed or underestimated. A correct diagnosis is necessary for proper management and genetic counseling.

1. Introduction

X-linked liver glycogenosis (XLG), also known as glycogen storage disease (GSD) type-IXa, results from a genetic deficiency of hepatic phosphorylase kinase (PHK) activity. PHK plays an important role in glycogen metabolism by activating glycogen phosphorylase for the degradation of glycogen to glucose-1-phosphate, and consists of four copies of four subunits each (α, β, γ and δ) to form a tetradecameric structure. The γ subunit harbors catalytic activity, which is activated by phosphorylation of the α and β subunits and the interaction of Ca^{2+} with the δ subunit. Mutations of *PHKA1* and *PHKA2* genes on the X chromosome, which encodes the muscle and liver isoforms of the α subunit, cause glycogen storage disease (GSD) type-IXc and type-IXa, respectively.[1] XLG can be divided into two subtypes: XLG-I, with a deficiency in PHK activity in peripheral blood cells and liver tissues; and XLG-II, with normal *in vitro* PHK activity in erythrocytes and leukocytes and low enzyme activity in the liver.[2,3] The differences between XGL-I and XGL-II led to the hypothesis that mutations in the

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PHKA2 gene in XLG-I disrupt the region of the α subunit that interacts with the catalytic subunit of PHK, whereas mutations in XLG-II might affect the hydrolytic activity of the PHK α subunit.[4]

The main symptoms of both types of XLG are hepatomegaly, growth retardation, elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, hypercholesterolemia, and hypertriglyceridemia. Unlike other types of GSD, XLG is generally a benign disease. Muscles with XLG are clinically and enzymatically normal, which is different from GSD type-IXc. The clinical symptoms gradually improve with age, and patients are often asymptomatic in adulthood.[5] Mutations of *PHKA2* have been reported in Western countries and Japan. However, there are no previous reports of XLG in the Chinese or Taiwanese population. Here, we report a family with XLG-I.

2. Case Reports

2.1. Case 1

A 4-year, 7-month-old boy, with a birth weight of 3,050 g and birth length of 50 cm, presented with abdominal distention and general malaise for 1 year. Physical examination revealed marked hepatomegaly, with a liver span of 7.5 cm below the right costal margin, and growth retardation, with a body height of 95 cm (3^{rd} to 10^{th} percentile), and a body weight of 17 kg (50^{th} to 75^{th} percentile). His development was normal, and neurological examinations revealed no abnormalities. He had been treated as non A-to-C hepatitis for 1.5 years, with fluctuating liver enzyme levels. His mother claimed no family history of hepatitis. The boy's hemogram was normal. Blood biochemistry showed elevated liver enzymes, with AST 317 U/L and ALT 362 U/L, triglycerides 170 mg/dL, total cholesterol 181 mg/dL, creatine phosphokinase (CK) 145 U/L, uric acid 5.5 mg/dL and normal bilirubin and blood sugar levels. There was no metabolic acidosis. Serum markers for viral hepatitis were all negative. Abdominal sonography revealed marked hepatomegaly, with a liver span of 11 cm at the right mid-clavicle line and 11 cm at the mid-sternal line, with homogeneously increased echogenicity. Liver histology (Figure 1A) showed diffuse clear cytoplasm in hepatocytes, which were positively stained for periodic acid-Schiff and negatively for diastase-resistant periodic acid-Schiff. Electron microscopic examination revealed glycogen filling in the cytoplasm of hepatocytes. Glycogen storage disease was diagnosed according to clinical and pathological findings. After a 4-year follow-up, the patient had mild hepatomegaly and intermittent mild elevation of ALT.

2.2. Case 2

A 1-year, 3-month-old boy was transferred to our hospital by his family physician due to hepatomegaly. His birth weight and height were 3,480 g and 49 cm, respectively, at a gestational age of 38 weeks. Growth retardation, with a body height of 75 cm (3^{rd}–10^{th} percentile) and a body weight of 9.9 kg (25^{th}–50^{th} percentile), was noted. Neurological development and examinations were normal. The parents denied any other affected individuals in the family. Blood biochemistry revealed abnormal liver enzymes, AST 138 U/L and ALT 151 U/L, triglycerides 83 mg/dL, total cholesterol 123 mg/dL, CK 110 U/L, uric acid 4.9 mg/dL and normal bilirubin and sugar levels. Hepatomegaly with homogeneous increased echogenicity was noticed on abdominal sonography. Liver histology and electron microscopy confirmed the diagnosis of glycogen storage disease (Figure 1B). After a 3-year follow-up, the patient's liver function tests became normal and mild hepatomegaly persisted.

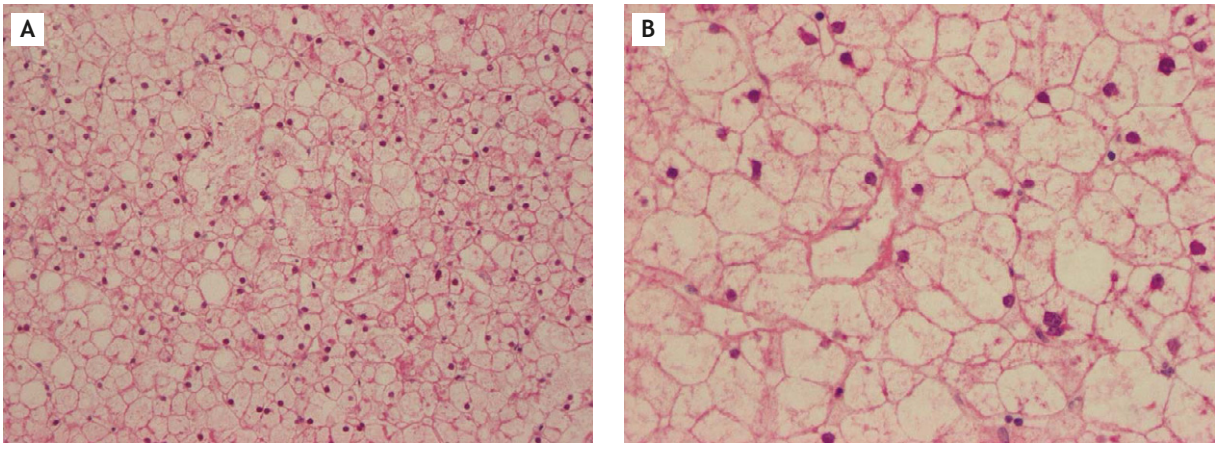

Figure 1 Liver histology of cases 1 (A) and 2 (B) show diffuse clear cytoplasm in the hepatocytes and hepatocyte ballooning change (hematoxylin and eosin; A: 40×; B: 200×).

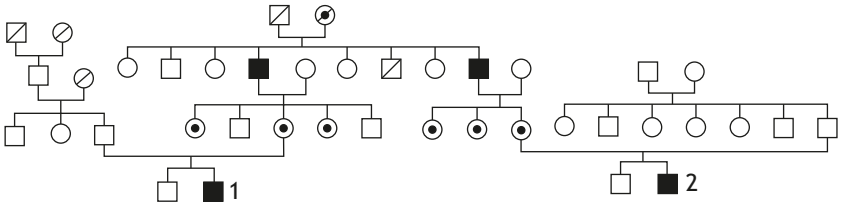

Figure 2 Pedigree of the two cases. Cases 1 and 2 were available for genomic analysis. Both maternal grandfathers of these patients (upper filled squares) were undiagnosed, according to their short stature and previous history of abnormal liver enzymes, suggesting an X-linked inheritance. ○ = female; □ = male; ⦿ = obligate female carrier; ■ = case; ⊘ ⊠ ⦻ = deceased.

2.3. Family history

Six months after Case 2 was diagnosed with glycogen storage disease, the mothers of both children, who were cousins, discovered that their children had similar diseases. A detailed pedigree was determined and suggested an X-linked inheritance (Figure 2). In addition to the two patients, the maternal grandfather of Case 2 had been diagnosed with hepatitis in childhood. His present body length was 150 cm, which is below the Taiwanese average range. Liver enzymes were normal at this time, and abdominal sonography showed negative findings. The maternal grandfather of Case 1 also had short stature at 150 cm, without history of liver disease.

2.4. PHK activity

The suspected X-linked inheritance of these two patients led us to consider type-IX GSD. Enzymatic tests of PHK activity on red blood cells and liver tissues in both patients were performed by the Duke University School of Medicine. Briefly, rabbit muscle phosphorylase was added as an exogenous substrate to detect endogenous PHK activity in erythrocytes and liver tissues. PHK activity deficiency could be detected by decreased phosphorylase ratios. In our cases, all tissues revealed no PHK activity compared with positive controls. Although we intended to perform enzyme assays for the two grandfathers, they refused to undergo the test.

2.5. *PHKA2* gene mutation analysis

Genomic DNA was isolated from peripheral blood leukocytes using the Puregene DNA isolation kit (Gentra, Minneapolis, MN, USA). PCR amplification of selected exons (exons 4, 6, 9, 26, 32 and 33) of the *PHKA2* gene (Genbank accession number NP_000283) was performed using the primer sets described in previous reports.[6] The PCR products were subjected to direct DNA sequencing. Sequence analysis was performed by dideoxynucleotide chain termination using the ABI 377 DNA Automated Sequencer (Applied Biosystems, Foster City, CA, USA). No mutations of these exons were noted in either patient.

3. Discussion

Both cases in this study presented with hepatomegaly, abnormal liver function and short stature, but no evidence of hypoglycemia. Both cases later showed spontaneous improvements in liver function. Therefore, a mild form of glycogen storage disease, such as GSD type-III or type-IX, was suspected. The family history strongly suggested an X-linked inheritance leading to a final diagnosis of XLG-I, which was confirmed by enzymatic tests. To our knowledge, this is the first report of Taiwanese patients with GSD type-IXa. Our report emphasizes the importance of establishing complete and detailed family history in diagnosing metabolic and genetic diseases. A detailed pedigree is needed to elucidate the inheritance patterns.

The main symptoms of XLG include hepatomegaly and abnormal liver enzyme levels. The clinical symptoms are generally benign. Hypoglycemia and metabolic acidosis, two symptoms that are frequently observed in most of the other types of GSD, are rarely seen in XLG. Initial growth retardation has been noted between the ages of 2–10 years. Subjects often approach normal height in adulthood.[7] Furthermore, clinical symptoms gradually disappear with age, leaving most adults asymptomatic. Because it is difficult to classify these patients, many have probably been diagnosed incorrectly. The prevalence rates are probably underestimated due to the benign presentation. No large-scale studies assessing the incidence and prevalence of XLG have been published. The two maternal grandfathers of our patients were suspected to be affected, because of the history of hepatitis and short stature. Because individuals with XLG may be asymptomatic in adulthood, it is difficult to reach a clinical diagnosis; the disease passes to descendents through undiagnosed males.

In the English literature, 43 patients with XLG-I and 16 with XLG-II have been reported to have 33 and 13 different PHKA2 gene mutations, respectively.[6,8–21] We failed to identify a mutation in our cases after sequencing exons 4, 6, 9, 26, 32 and 33, which cover 45.5% (15/33) of the reported mutation sites and 48.8% (21/43) of cases with XLG-I. The genetic defects in our cases may be located in

uncommon sites that warrant further investigation. It is possible that ethnic groups have diverse mutations because different mutations have been noted in Dutch, French and Japanese patients.

Some types of GSD have been reported to be complicated by hepatic adenoma, hepatocellular carcinoma, acute myelogenous leukemia and neutropenia during follow-up.[22–24] No associated diseases or malignancies have been reported with type IX GSD. Most asymptomatic patients with XLG are undiagnosed. Long-term follow-up of our patients is necessary to monitor and determine regression in liver disease with age and any associated metabolic complications.

3. Conclusions

In summary, we report the first family diagnosed with XLG-I in Taiwan. Careful collection of clinical and pathological evidence, as well as detailed family histories, is essential for diagnosis. Because this is a relatively benign form of GSD, many cases are probably undiagnosed and the prevalence rate is therefore underestimated. XLG should be considered in cases of idiopathic hepatopathy and short stature in children or adults. Further genetic studies to identify the mutations in Taiwanese patients should be performed to confirm the molecular basis for these patients.

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